

App. No. 10/590,552  
Office Action Dated July 7, 2009

### REMARKS

Favorable reconsideration is respectfully requested in view of the above amendments and following remarks. Claim 56 has been amended. The amendment to claim 56 is supported by the original disclosure, for example by page 19, line 26 to page 20, line 6. Claim 62 has been amended editorially. Claim 73 has been canceled without prejudice or disclaimer. Claims 56, 59-63, 66, 69 and 72 are pending. No new matter has been added.

#### *Election/Restrictions*

The Examiner's justification for restriction is that the preamble of claim 56 is directed to a species of "organochlorine compound" (i.e., PCE or TCE) while the body of the claim is directed to a genus comprising "organochlorine compound". However, when limitations in the body of the claim rely upon and derive antecedent basis from the preamble, then the preamble may act as a necessary component of the claimed invention. *See, e.g., Eaton Corp. v. Rockwell Int'l Corp.*, 323 F.3d 1332, 1339, 66 U.S.P.Q.2d 1271, 1276 (Fed. Cir. 2003); *Electro Sci. Indus. v. Dynamic Details, Inc.*, 307 F.3d 1343, 1348, 64 U.S.P.Q.2d 1781, 1783 (Fed. Cir. 2002); *Rapoport v. Dement*, 254 F.3d 1053, 1059, 59 U.S.P.Q.2d 1215, 1219 (Fed. Cir. 2001). In claim 56, the term "organochlorine compound" is first recited in the body in line 8, and derives antecedent basis from the preamble by reciting "the" before "organochlorine compound". Therefore, the limitation "organochlorine compound that is at least one of tetrachloroethylene (PCE) and trichloroethylene (TCE)" should be accorded patentable weight. Deneff et al. discloses detecting the specific aromatic oxygenase genes in a soil community degrading polychlorinated biphenyls (PCBs). Deneff et al. do not disclose judging a biological in an environment contaminated with an organochlorine compound that is at least one of tetrachloroethylene (PCE) and trichloroethylene (TCE). Accordingly, Applicants respectfully submit that claim 56 provides the special technical feature common to all claims and that the restriction requirement is improper. Withdrawal of the restriction/election of all claims is requested.

#### *Information Request*

The Examiner has requested further information regarding the poster at the Society for Biotechnology, Japan in 2003 (Scott et al., Construction of a 16S-23S ribosomal DNA internal transcribed space sequence based microarray for detection of PCE degrading

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microorganisms). Applicants submit herewith the material used by Mr. Randolph Scott in the poster presentation at the Society for Biotechnology, Japan in 2003.

***Claim Objections***

Claim 56 is objected to because of informalities. Claim 56 has been amended, taking the issues noted in the objection into account. Withdrawal of the objection is respectfully requested.

***Claim rejections - 35 U.S.C. § 102/103***

Claims 56, 59 and 60 are rejected under 35 USC 102(b) as being anticipated by, or in the alternative, under 35 USC 103(a) as obvious over Scott et al. (The Society for Biotechnology, Japan, 55<sup>th</sup> meeting 2003). Applicants respectfully traverse the rejection.

Claim 56 is directed to a method of judging biological activity in an environment contaminated with a organochlorine compound that is at least one of PCE and TCE. Claim 56 recites the use of a DNA probe that is a combination of 87 types of DNA probes, each of which is bonded specifically to an internal transcribed spacer region of any of the 17 types of bacteria denoted as A to Q without occurrence of cross-hybridization. Advantageously, the method allows the detection of any of the 17 types of bacteria having biological activity of degrading PCE not only simultaneously, but with very high level of reliability.

Scott et al. disclose 18 bacteria associated with the PCE degradation pathway. Scott et al. disclose that several unique 40-mer 50% GC probes having minimum self-complementarity were used to detect and distinguish the 18 bacteria. However, Scott et al. do not disclose the sequence of the probes, or the sequence of the primers that were used to obtain the probes. Moreover, nothing in Scott et al. teaches or suggests that the probes that were obtained could be combined and used as a single DNA probe and detect any one of the 17 types of bacteria denoted as A to Q without any occurrence of cross-hybridization as recited in claim 56.

The rejection contends that it would have been obvious to use the 16S-23S-ITS region sequence from 'A' to 'Q' bacteria and design the probe comprising SEQ ID Nos. 19-105 for judging a biological activity in an environment contaminated with PCE as taught by Scott et al. Applicants submit that while the control of experimental parameters and design of a probe is less complicated where a probe including only several types of probes are used per hybridization, the art remains unpredictable as to the design of probes that are both highly

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sensitive and highly specific (i.e., bind to the target without occurrence of cross-hybridization) where more than a few types of probes are used, and especially where more than one type of closely related species are tested per hybridization. That is, in general, the likelihood of achieving probes with both high sensitivity and high specificity is inversely proportional to the number of types of probes and species to be tested, and how close the species are related. Claim 56 recites the use of a DNA probe that is a combination of well over several types of probes, namely 87 types of DNA probes. Further, according to the features of claim 56, the DNA probe can test as many as 17 types of bacteria that are phylogenetically related and are associated with PCE bioremediation. Even further, each of the 87 types of DNA probes are capable of binding to the ITS region of any of the 17 types of bacteria without occurrence of cross-hybridization. Scott et al. fail to provide any experimental data or any guidance as to achieving a DNA probe that is a combination as many as 87 types of DNA probes, where each of the 87 types of DNA probes are capable of binding to the ITS region of any of the 17 types of bacteria without occurrence of cross-hybridization as recited in claim 56 with a reasonable expectation of success. Therefore, claim 56 and its dependent claims are patentable over Scott et al.

Claims 56, 59 and 60 are rejected under 35 USC 103(a) as being unpatentable over Ebersole et al. (US 2003/0077601) in view of Buck et al. (Bio Technique, 1999, pp. 528-536). Applicants respectfully traverse the rejection.

The rejection contends that it would have been obvious to combine Ebersole and Buck et al. and achieve the features of the claims. However, the references fail to provide any reason to expect that a DNA probe including 87 types of DNA probes could be used to detect the degrading capability of the 17 types of bacteria, where each of the 87 types of DNA probes is capable of binding specifically to an internal transcribed spacer region of any one of the 17 types of bacteria without occurrence of hybridization.

In particular, Ebersole teaches the design of primers and probes to detect 'A', 'H', 'K' and 'M' by aligning the dechlorinating 16S rRNA sequences of the bacterium and determining the variable sequences within the dechlorinating 16S rRNA sequences (Example 9, paragraphs [0215] to [0219]). Ebersole clearly is focused on finding unique sequences within a functionally related region, and is silent as to a DNA probe that binds specifically to an internal transcribed spacer region. Ebersole also fails to provide any experimental data or

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any guidance as to whether any of the probes are capable of binding to the target without occurrence of cross-hybridization. On the other hand, claim 56 recites that each of the 87 types of DNA probes is capable of binding specifically to an ITS region of any of the 17 types of bacteria without occurrence of cross-hybridization.

Buck does not remedy the deficiencies of Ebersole. In particular, the rejection recognizes that Ebersole does not teach the claimed probes with SEQ ID Nos. 19-105 and 116 and 117, but contends that since Buck teaches any primer will amplify the nucleic acid sequence of interest, one would be motivated to use an alternative primer set and achieve the claimed probes with a reasonable expectation of success. However, Buck merely teaches that high quality products may be achieved using various primers under ideal conditions (see, e.g., paragraph bridging pages 535-6), and is silent as to whether the resulting probes themselves would bind specifically to a given region without occurrence of cross-hybridization, let alone specifically teach a DNA probe including 87 types of DNA probes that could be used to detect the degrading capability of 17 types of bacteria A to Q, where each of the 87 types of DNA probes is capable of binding specifically to an internal transcribed spacer region of any one of the 17 types of bacteria without occurrence of hybridization. Accordingly, Ebersole and Buck fail to provide any experimental data or any guidance as to whether the features of claim 56 could be achieved with a reasonable expectation of success.

In view of the above, favorable reconsideration in the form of a notice of allowance is requested. Any questions or concerns regarding this communication can be directed to the attorney-of-record, Douglas P. Mueller, Reg. No. 30,300, at (612) 455.3804.

**52835**

PATENT TRADEMARK OFFICE

Dated: *Oct 7, 2009*

Respectfully submitted,

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#### Summary of Presented Results in Kumamoto Conference:

#### Detection of PCE-degrading bacteria from soil and water samples from Matsushita

##### METHODS:

A 20 g soil (16 September 2002) and a 300-ml water (10 September 2003) sample was obtained from Matsushita on separate occasions. DNA from 250 mg soil sample was extracted by using a FastPrep bead-beater and a soil DNA extraction kit as per instructions on the kit. The water sample was centrifuged at 7,000 rpm and then the pellet consisting of soil and cell debris was subjected to the same soil extraction and bead-beating procedures above. Approximately 1 ul of each sample was added to 50-ul standard Amplitaq Gold PCR mixes containing regular 27F and Cy3-labeled 132R primers. PCR was done according to standard protocol. The PCR products were desalted by Autoseq G-50 and vacuum-dried by SpeedVac. The dried PCR products were dissolved with a buffer containing a final concentration of 5x SSC, 0.2% SDS and 50% formamide. The dissolved PCR products were boiled at 94°C for 3 min and then cooled in ice for less than two minutes and applied to the surface of the microarray slides. Cover glass slips were then applied onto the microarray and the complete hybridization setup was placed in a hybridization chamber which was then placed in 42°C for at least 4 hours. Microarray slides were then washed with 0.2xSSC 0.2% SDS for 5 minutes, 0.2xSSC for 5 minutes and then 0.05xSSC for several seconds and spin-dried at 1,800 rpm. The microarray slides were then scanned using Scanarray version 5.

##### RESULTS:

Some, but not all of the spots corresponding to M bacteria (*Dehalobacter restrictus*) probes gave significant positive hybridization for the soil sample PCR products (see Figure 1) while some, but not all spots corresponding to J bacteria (*Clostridium formicoaceticum*) probes gave weak but significant hybridization for the water sample PCR products (see Figure 2).

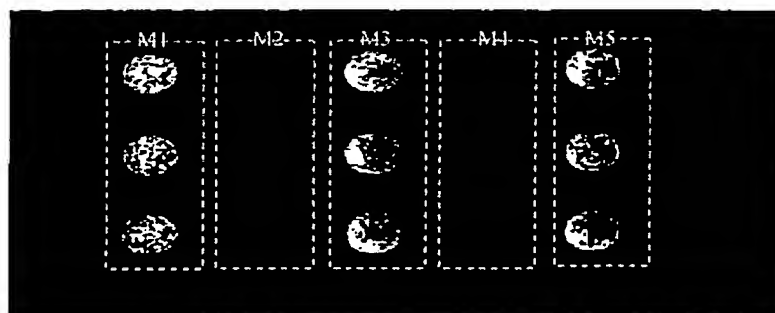
Right at this moment the positively-hybridizing PCR product for the soil sample has been cloned and will be sequenced in the future to confirm if it is the one we are looking for. On the other hand the water sample PCR product that gave positive spots on the microarray has to be cloned and sequenced to confirm its identity.

The water sample has been analyzed in Prof. Takamizawa's laboratory in Gifu University (see Table) and it showed a relatively high amount of cis-DCE and but no PCE

(tetrachloroethylene). We are expecting other kinds of bacteria also present in the water samples. since *C. formicoaceticum* alone can only convert PCE to TCE. There must be other bacteria present that converted PCE or TCE to cis-DCE and other compounds and it will be analyzed by further dilutions of PCR reactions as well as cloning. The soil sample, however, must also be analyzed for volatile organic compounds (PCE, TCE, cisDCE, etc) as well.

FIGURE 1

DETECTION OF M BACTERIA (Dehalobacter  
restrictus) FROM A CONTAMINATED SOIL  
SAMPLE:



Contaminated soil sample hybridization

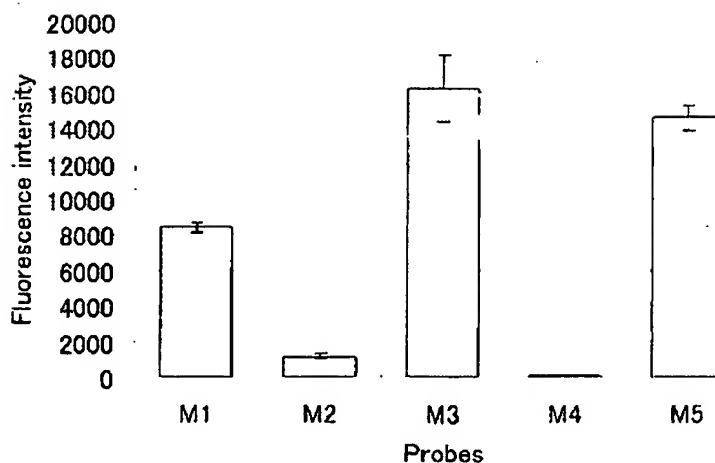
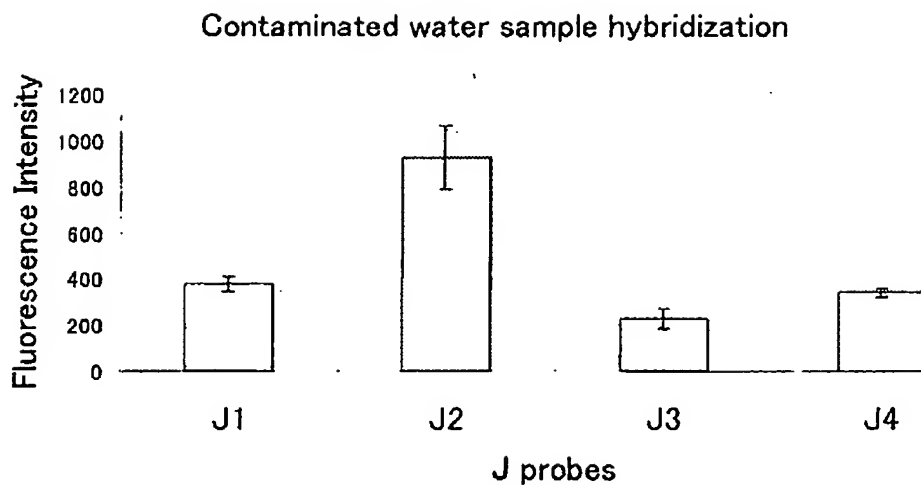
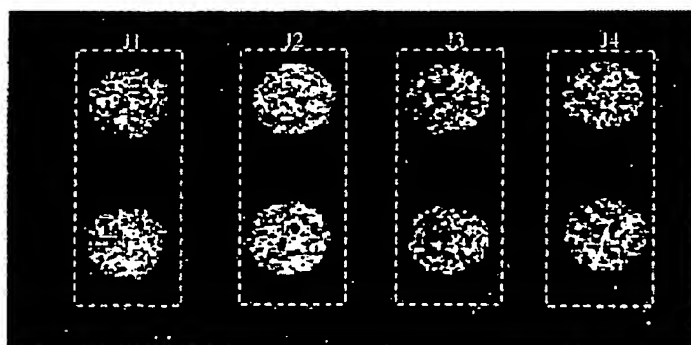


FIGURE 2

DETECTION OF J BACTERIA (*Clostridium*  
*formicoaceticum*) FROM A CONTAMINATED  
WATER SAMPLE:



## TABLE

## CONCENT OF VOLATILE CHLORINATED COMPOUNDS OF WATER SAMPLE

NAME OF COMPOUND	CONCENTRATION (ppm)
Tetrachloroethylene	Not detected
Trichloroethylene	0.012
cis-1,2-Dichloroethylene	3.34
trans-1,2-Dichloroethylene	0.115
1,1-Dichloroethylene	0.012
Vinyl chloride	0.212

## RECOMMENDATIONS:

I would recommend to get more of the water sample of the same source but of slightly different position or location—the upstream source of contamination should as well be sampled for soil and water since there is where the probable bioremediation is beginning or occurring.



# PCE MICROARRAY:

**Construction of a  
16S-23S ribosomal DNA  
internal transcribed spacer sequence  
(16S-23S rDNA ITS)-based microarray  
for the detection of perchloroethylene  
(PCE)-degrading microorganisms**

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Scott, R., Gifu University

Takamizawa, K., Ph.D., Gifu University

Iwahashi, H., Ph.D., National Institute of Advanced  
Industrial Science and Technology (AIST), Tsukuba

## BIOREMEDIATION OF PERCHLOROETHYLENE:

- PCE as well as TCE are toxic groundwater pollutants from dry cleaning and other industries
- can cause cancer
- PCE can be degraded by dehalorespiration of anaerobic bacteria
- PCE can be removed efficiently if bacteria are present/introduced in the contaminated sites

## DNA-BASED METHODS OF BACTERIAL DETECTION:

- 16S rDNA clone library sequencing
- *In situ* hybridization
- detection by PCR
- ribotyping analysis

→ Microarray analysis

## OBJECTIVES:

- To construct an ITS-based oligonucleotide microarray for the detection of perchloroethylene-degrading anaerobic bacteria
- To evaluate the microarray for the actual detection of bacteria in a typical contaminated site

## 18 BACTERIA ASSOCIATED WITH PCE DEGRADATION:

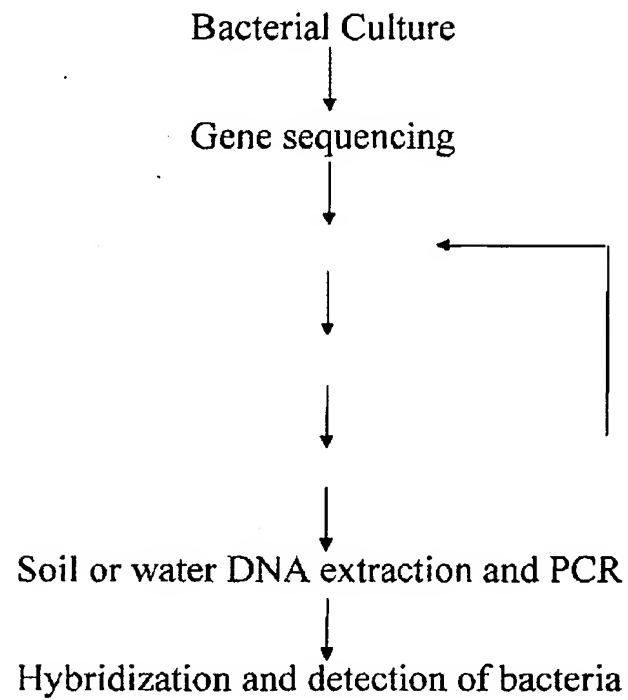
<i>Dehalococcoides ethenogenes</i> 195 R	PCE → TCE → DCE → VC → ethene
<i>Desulfitobacterium frappieri</i> B	PCE → TCE → cisDCE
<i>Desulfitobacterium hafniense</i> I	
<i>Desulfitobacterium dehalogenans</i> H	
<i>Desulfitobacterium</i> sp. strain PCE1 N	
<i>Desulfitobacterium frappieri</i> TCE1 O	
<i>Desulfomonile tiedjei</i> DCB-1 Q	
<i>Desulfuromonas chloroethenica</i> K	PCE → TCE → DCE
	PCE → TCE
<i>Clostridium formicoaceticum</i> J	PCE → TCE
<i>Dehalobacter restrictus</i> M	PCE → cisDCE
<i>Dehalospirillum multivorans</i> A	PCE → cisDCE
<i>Desulfomicrobium norvegicum</i> G	PCE → cisDCE
	DEC, VC → CO <sub>2</sub>
<i>Xanthobacter flavus</i> E	DCE, VC → CO <sub>2</sub>
<i>Mycobacterium</i> L1 F	VC → CO <sub>2</sub>

## SEQUENCE IDENTITIES OF DESULFITOBACTERIUM SPECIES :

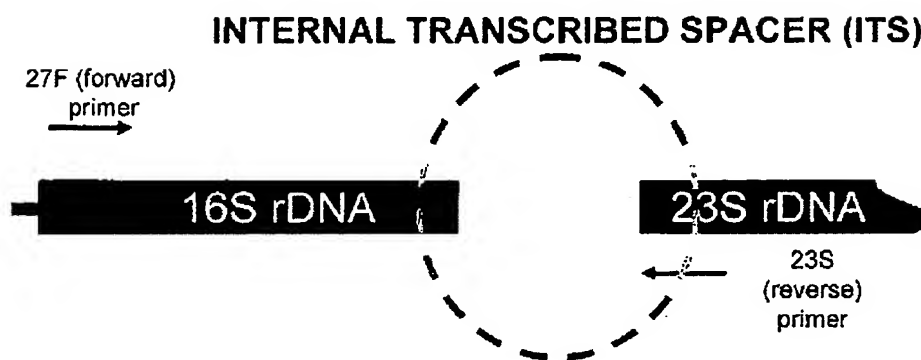
	D. frap pieri	D. dehaL ogenans	D. haf- niense	D. PCE1	D. TCE1
D. frap pieri	1.00	0.95	0.92	0.83	0.78
D. deha logen- ans	0.67	1.00	0.95	0.83	0.78
D. haf- niense	0.64	0.69	1.00	0.83	0.78
D. PCE1	0.64	0.83	0.78	1.00	0.78
D. TCE1	0.57	0.51	0.43	0.49	1.00

16 S rDNA sequence identity

## METHODOLOGY:



## ITS SEQUENCING:



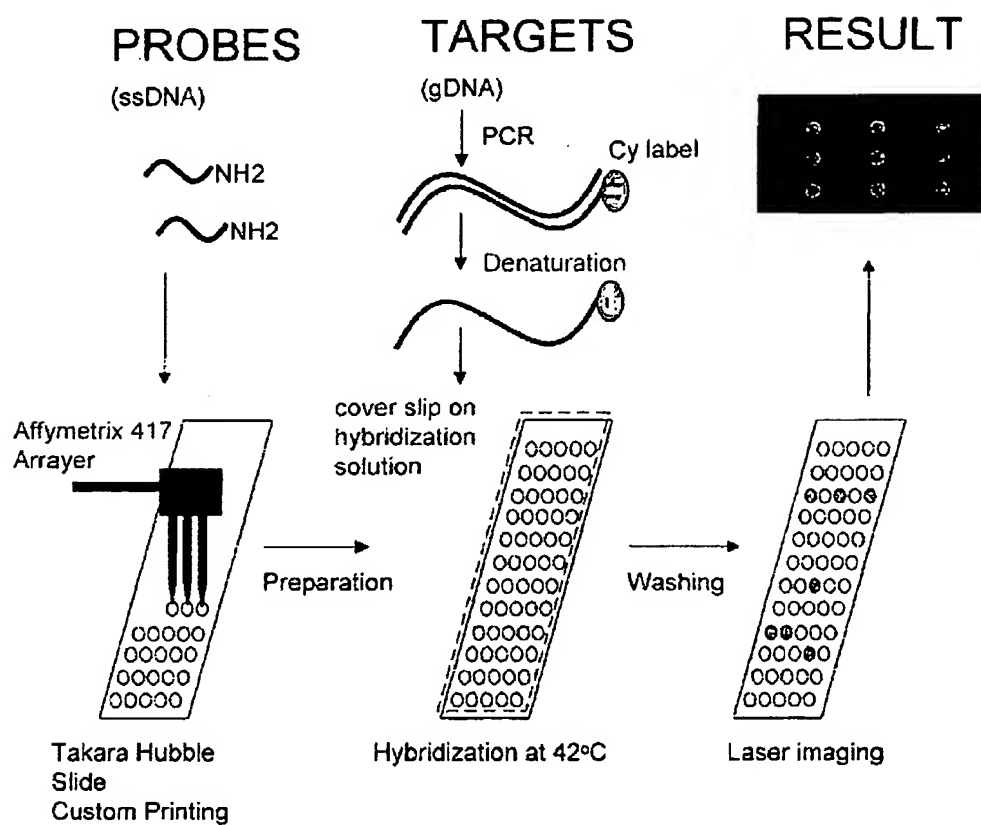
The ITS sequence was  
used for probe  
construction



## PROBE DESIGN:

- 40-mer, 48-50% GC, single-stranded probes
- little or no self-complimentarity (no hairpins)
- no hits in GenBank (2 or more mispairs)
- 2-7 unique probes were chosen for each bacteria

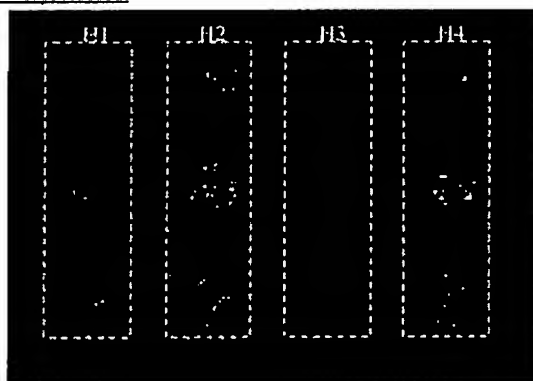
## PCE MICROARRAY:



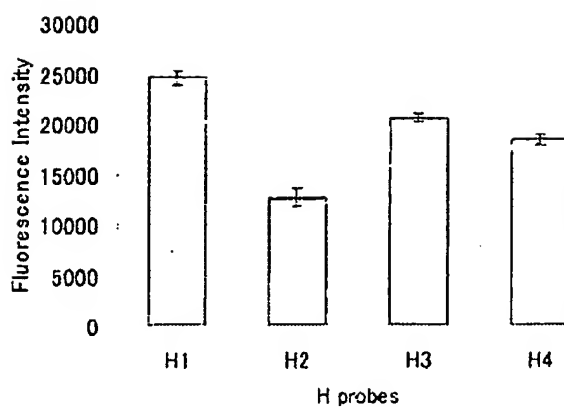
## MICROARRAY QUALITY PARAMETERS:

- Spot reproducibility and low background intensity
- Specificity
- Sensitivity
- Actual detection of bacteria from soil or water sample

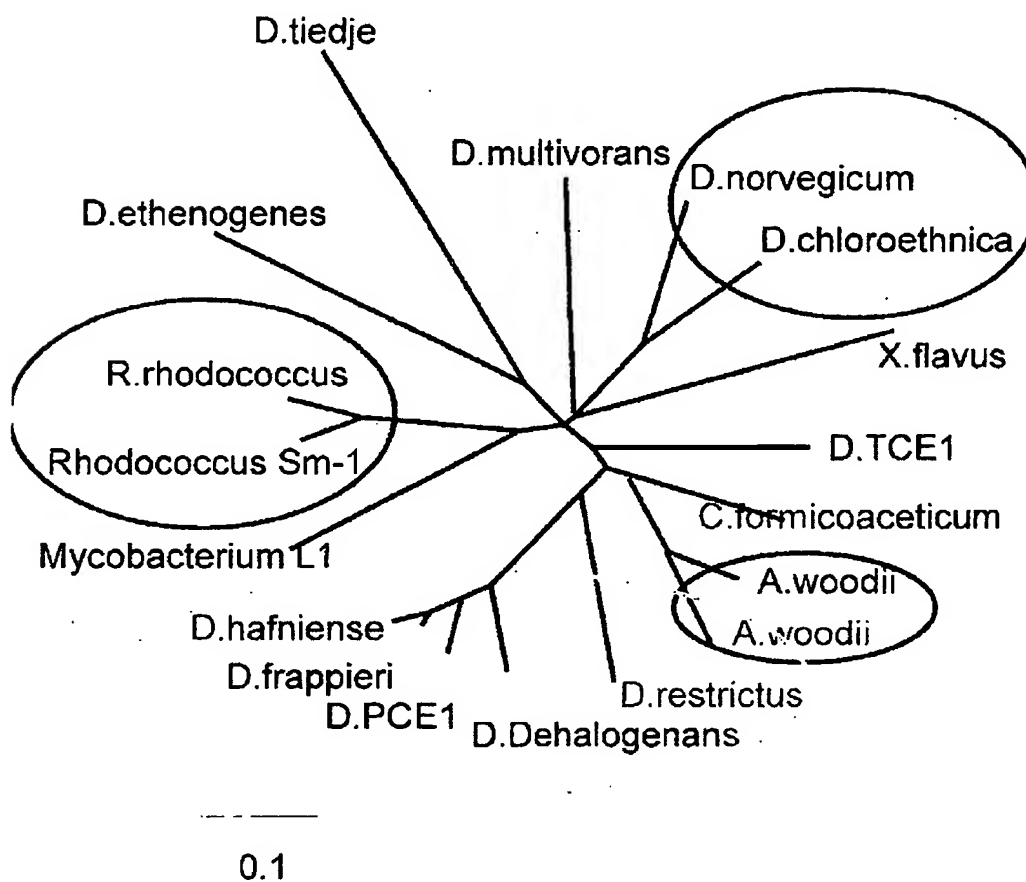
## A. SPOT REPRODUCIBILITY AND LOW BACKGROUND INTENSITY:



Hybridization to H probes

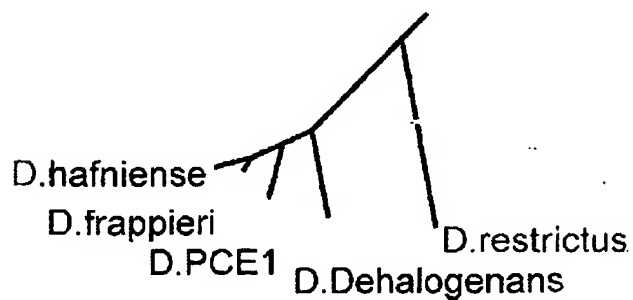
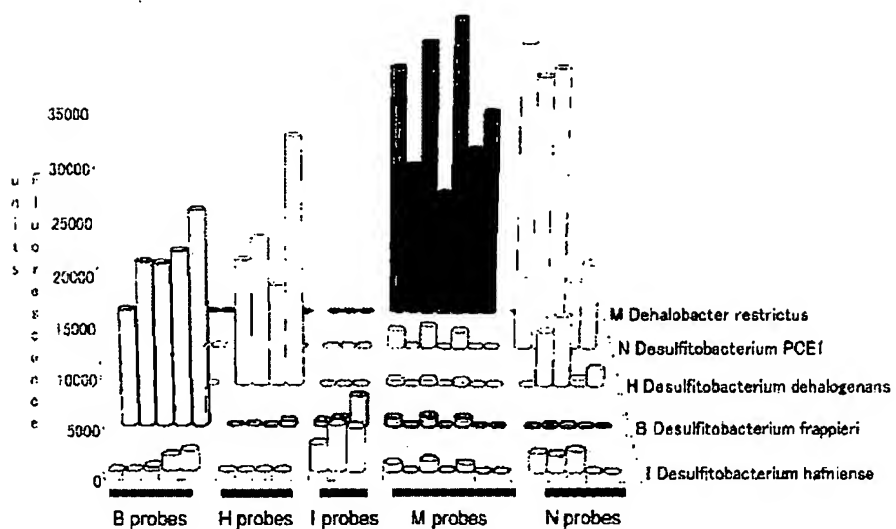


## ITS PHYLOGENETIC TREE:



## B. SPECIFICITY:

B, H, I, N and M Probes Specificities



### C. SENSITIVITY:



1/10th dilution  
=500 ng total



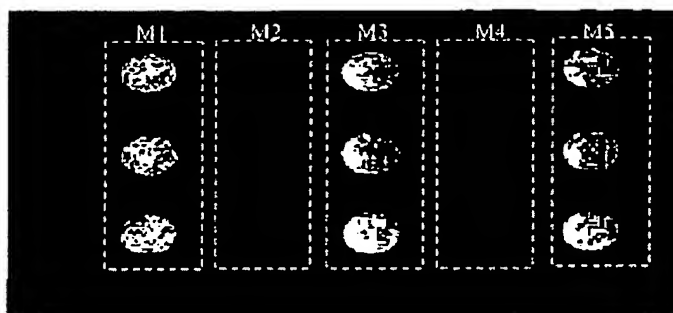
1/100th dilution  
=50 ng total



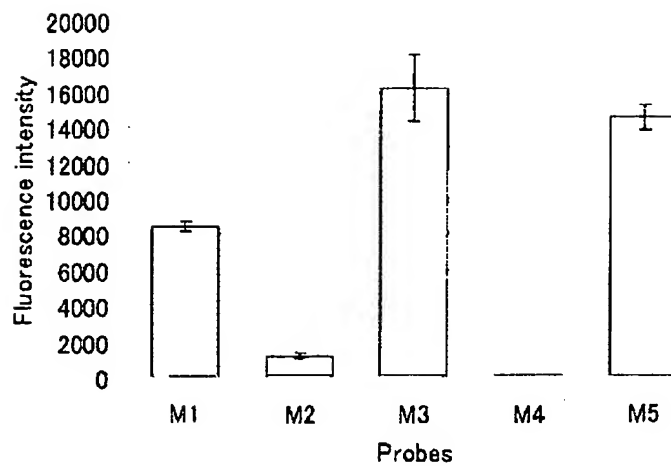
1/1000th dilution  
=5 ng total

Hybridization to H probes at different  
dilutions of Cy3-labeled target

## D. DETECTION OF BACTERIA FROM A CONTAMINATED SOIL SAMPLE:

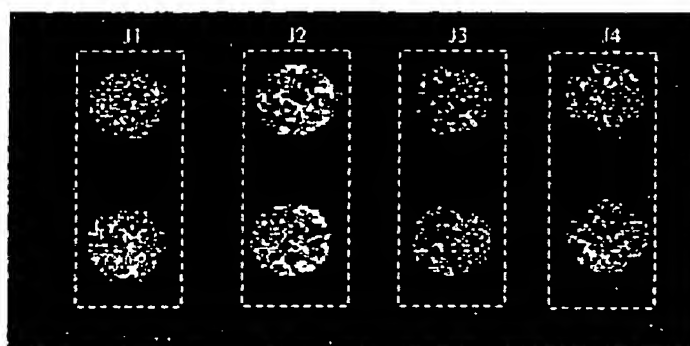


Contaminated soil sample hybridization

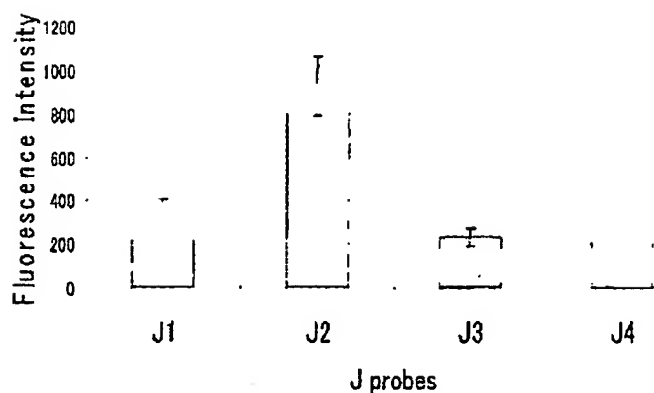




## D. DETECTION OF BACTERIA FROM A CONTAMINATED WATER SAMPLE:



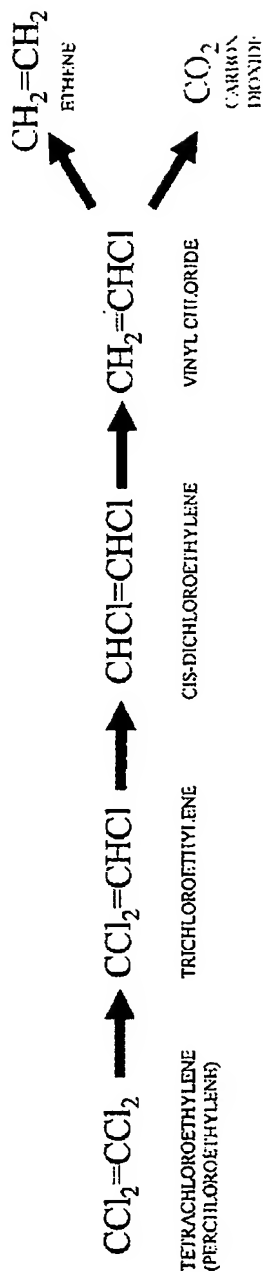
Contaminated water sample hybridization



## CONCLUSIONS:

1. An ITS-based 40-mer oligonucleotide microarray can detect and differentiate all 18 PCE-degrading anaerobic bacteria even up to the subspecies level
2. The ITS microarray was shown to detect two kinds of bacteria from a contaminated soil and water sample

# LIST OF BACTERIA ASSOCIATED WITH THE PCE DEGRADATION PATHWAY



- Desulfotobacterium frappieri* B
- Desulfotobacterium hafniense* I
- Desulfotobacterium dehalogenans* H
- Desulfotobacterium* sp. strain PCE1 N
- Desulfotobacterium frappieri* TCE1 O
- Desulfomonile tiedjei* DCB-1 Q
- Desulfomonas chloroethenica* K
- Dehalobacter restrictus* M
- Dehalospirillum multivorans* A
- Desulfomicrobium norvegicum* G
- Clostridium formicoaceticum* J

- Rhodococcus* sp. Sm-1 C
- Rhodococcus rhodococcus* D
- Xanthobacter flavus* E